TABLE I.
Viability of Minced Tumor after Freezing and Storage at —65° to —76°C.

| Donor | Transfer No. | Tumor incidence T/I* | | | Storage period (days) |
|---|---|---|---|---|---|
| | | Fresh inoculum not frozen | Inoculum frozen only | Inoculum frozen and stored | |
| I | 1 | 12/12 | — | 2/6 | 28 |
| II | 3 | — | — | 3/3 | 10 |
| III | 4 | 12/12 | — | 7/7 | 182 |
| | | | | 7/7 | 391 |
| IV | 4 | 1/4 | 2/4 | 8/8 | 384 |
| V | 6 | 3/3 | — | 2/7 | 369 |
| VI | 10 | 4/5 | — | 7/7 | 328 |
| VII | 12 | 4/4 | 3/3 | 8/8 | 102 |
| | | | | 2/7 | 307 |
| VIII | 18 | 3/3 | — | 7/8 | 245 |
| IX | 20 | 4/4 | — | 8/8 | 225 |

*T—No. of birds that developed a tumor at site of inoculation.
I—No. of birds inoculated.

Samples tested after freezing and storing for 10 to 391 days in a $CO_2$ icebox produced 100% takes in 7 of 11 tests. Two inoculations showed an apparent increase in activity; whereas, 4 tests showed a decrease after storage. One of these, sample VII, produced tumors in all of the 8 birds inoculated after 102 days of storage but only 2 of 7 birds developed tumors after 307 days of storage. However, there appeared to be no definite relation between the length of period stored and loss in activity. The minced tumors that showed a decrease in activity were among those stored in Lusteroid tubes, while those stored in pyrex glass produced takes in all birds inoculated with tumor stored up to 391 days. It may be possible that inadequate seals were obtained with screw cap and tape and thus sufficient $CO_2$ was allowed to enter some of the Lusteroid tubes and partially inactivate the minced tumor.

Tumors obtained from recipients of 4 of the storage tests described were transferred in one case in the fresh unfrozen state, and in 3 other cases after the tumors had been frozen and stored for 22 to 229 days.

Typical tumors developed in all of 34 birds inoculated. Thus, the storage of minced tumor tissue at low temperatures has no apparent effect upon its ability to produce typical tumors after one passage in birds or upon its viability during low temperature storage after one transfer in birds.

*Conclusion.* When the transmissible fowl tumor (Olson) was frozen slowly and stored at −65°C to −76°C for 10 to 391 days, no significant reduction or alteration in its capacity to produce typical tumors in the pectoral muscle was detected by methods employed.

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## "Folic Acid" a Tumor Growth Inhibitor.

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Studies on tumor growth inhibitors, carried out in this laboratory, suggested the possible importance of "folic acid" for malignant growth. Evidence has recently been presented that inositol, another member of the vitamin B-complex, inhibited tumor growth, whereas 8 other crystalline B-vitamins were ineffective.[1]

In the following we report experiments in which the action of a "folic acid concentrate"*

and the action of the crystalline *L. casei* factor[2]† on tumor growth was investigated. In these studies a rapid test for tumor growth inhibitors was employed, as previously described.[3] In this test the inhibitory action is judged by comparing tumor sizes and tumor weights of matched treated and untreated groups of mice within an experimental period of 48 hours.

In Table I one experiment is presented, in which varying doses of the "folic acid concentrate" were studied. In Table II one experiment is presented, in which varying doses of *L. casei* factor were studied.

It is evident from these tables that "folic acid concentrate" and *L. casei* factor are strong inhibitors of tumor growth. In these experiments 0.16 γ "folic acid concentrate" and 0.025 γ *L. casei* factor inhibited tumor growth significantly in a group of 7 mice. Similar results were obtained with "folic acid concentrate" in 6 experiments on 117 mice and with *L. casei* factor in 10 experiments on 364 mice. On weight basis, the *L. casei* factor is a many hundredfold stronger inhibitor than inositol.

TABLE I.

Effect on Tumor Growth of 4 Intravenous Injections of "Folic Acid Concentrate" in Varying Doses Given Over a Period of 48 Hours.* Seven animals were used in each group.

| Group No. | Dose of "folic acid"† γ | Mean terminal tumor wt, mg | Standard error, mg |
|---|---|---|---|
| 805 | 0 | 951 | 88 |
| 803 | 0.16 | 616 | 16 |
| 802 | 0.32 | 566 | 57 |
| 801 | 0.63 | 509 | 47 |
| 800 | 1.25 | 470 | 45 |
| 799 | 2.50 | 386 | 42 |
| 798 | 5.00 | 333 | 67 |

* Female Rockland mice transplanted with Sarcoma 180; start of the experiment 8 days after transplantation; mice kept on normal diet.

† Micrograms of material of "potency" 40000 (Williams standard, as measured by *L. casei* assay.)

TABLE II.

Effect on Tumor Growth of 4 Intravenous Injections of *L. casei* Factor in Varying Doses Given Over a Period of 48 Hours. Seven animals were used in each group.

| Group No. | Dose of *L. casei* factor γ | Mean terminal tumor wt, mg | Standard error, mg |
|---|---|---|---|
| 828 | 0 | 893 | 78 |
| 827 | .0063 | 704 | 37 |
| 826 | .0125 | 650 | 60 |
| 825 | .0250 | 540 | 23 |
| 824 | .0500 | 517 | 36 |
| 823 | .1000 | 469 | 48 |
| 822 | .2000 | 394 | 21 |
| 821 | .4000 | 333 | 18 |

* Female Rockland mice transplanted with Sarcoma 180; start of the experiment 8 days after transplantation; mice kept on normal diet.

Pollack, Taylor, and Williams[4] reported that the vitamin present at the highest relative level in both human and rat cancer (the same seems to be true for the mouse) is folic acid. According to their Table 14 the relative inositol level is next to that of folic acid, while pyridoxine and biotin are present at relatively low levels. It seems of interest to investigate the possible relationship between the inhibitory action of folic acid and inositol and their relatively high content in malignant tumors.

*Summary.* A "folic acid concentrate" and a crystalline *L. casei* factor were found to be strong inhibitors of tumor growth.

1 Laszlo, D., and Leuchtenberger, C., *Science*, 1943, **97**, 515.

* We received the "folic acid concentrate" through the courtesy of Dr. J. C. Keresztesy of the Research Laboratories of Merck and Co., Inc.

2 Stokstad, E. L. R., *J. Biol. Chem.*, 1943, **149**, 573.

† The *L. casei* factor was obtained through the courtesy of Dr. B. L. Hutchings and Dr. E. L. R. Stokstad of Lederle Laboratories, Inc.

3 Laszlo, D., and Leuchtenberger, C., *Cancer Research*, 1943, **3**, 401.

4 Pollack, M. A., Taylor, A., and Williams, R. J., *Studies on B Vitamins in Human, Rat, and Mouse Neoplasms*, The University of Texas Publication, 1942, 4237, 56.